



Characterisation of Interleukin-8 and Monocyte Chemoattractant Protein-1 Expression within the Abdominal Aortic Aneurysm and their Association with Mural Inflammation

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Interleukin-8;
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Inflammation

Abstract *Objectives:* Abdominal aortic aneurysms (AAAs) are characterised by chronic transmural inflammation. This study investigated the expression of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) within the AAA, and their relationship with mural inflammation.

Methods: Biopsies were obtained from 25 AAAs, 15 abdominal aortas, and 10 atherosclerotic thoracic aortas. IL-8 and MCP-1 expression was measured in homogenised specimens by ELISA. Infiltrate composition and localised expression of IL-8 and MCP-1 were determined through immunohistochemistry.

Results: ELISA analysis demonstrated that IL-8 and MCP-1 were raised in the AAA compared to the controls [(IL-8, AAA vs. abdominal aorta: >28-fold, $P < .001$; AAA vs. thoracic aorta: >28-fold, $P < .001$) (MCP-1, AAA vs. abdominal aorta: 9-fold, $P < .001$; AAA vs. thoracic aorta: 19-fold, $P < .001$)]. Immunohistochemistry revealed that IL-8 was localised to the inflammatory infiltrate, which consisted predominantly of CD3⁺ T- and CD20⁺ B-lymphocytes. MCP-1 was predominantly expressed by CD68⁺ macrophages. Increasing IL-8 expression was associated with an increase in mural inflammation, and an increase in CD3⁺ T-lymphocytes of CD4⁺ phenotype within the infiltrate population.

Conclusion: Pathways involving IL-8 and MCP-1 may be involved in AAA pathogenesis. IL-8 may be directly involved in the chemotaxis of T_H-lymphocytes into the AAA wall.

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Introduction

The abdominal aortic aneurysm (AAA) is characterised by extensive extracellular matrix remodelling of the aortic wall, and is associated with increased mural proteolytic activity and inflammation. Extracellular matrix changes include an extensive degradation of elastin, increased collagen metabolism and invasion of the adventitia by a prominent inflammatory infiltrate.^{1–4}

Lymphocytes and macrophages form the major cellular component of the inflammatory infiltrate.^{4,5} These inflammatory cells are implicated in AAA pathogenesis through the secretion and activation of matrix metalloproteinases, which are capable of degrading elastin and other matrix components.^{6,7} However, the stimuli for the migration of these cells into the AAA wall are unknown. The directional movement of inflammatory cells into injured tissue is often induced by chemotactic proteins known as chemokines.⁸ Chemokines represent a superfamily of cytokines and are potent chemoattractants and activators of specific leukocytes *in vivo*.

Previous studies on cytokine expression within the aneurysm have demonstrated an overall pro-inflammatory, chemotactic environment within the aneurysm wall.^{9–11} In an earlier study we screened the AAA for the expression of 42 cytokines and found that several chemokines were raised within the human AAA wall, with the most significant increases in interleukin-8 (IL-8) and monocyte chemoattractant protein (MCP) expression.¹²

IL-8 is an 8.4 kDa, pro-inflammatory chemokine from the CXC chemokine family and signals through the receptors, CXCR1 and CXCR2. The main biological function of IL-8 involves neutrophil chemotaxis and degranulation.^{13,14} However, IL-8 is also chemotactic to other leukocytes, and has been shown to stimulate angiogenesis by promoting proliferation and chemotaxis of endothelial cells.^{15,16} MCP-1 belongs to the CC chemokine family and signal through the CCR2 receptor. MCP-1 chemoattracts and activates monocytes, but also stimulates T-lymphocytes, basophils, natural killer cells, dendritic cells and vascular SMCs.¹⁷

As potent chemoattractants the expression of IL-8 and MCP-1 within the AAA wall may be involved in the recruitment and/or maintenance of the inflammatory infiltrate. However, the relationship between these chemokines and mural inflammation remains to be determined. The aim of this study therefore was to elucidate the chemotactic stimuli that may orchestrate leukocyte recruitment into the outer aortic wall. This study describes the localised expression of IL-8 and MCP-1 within the AAA wall and the association between the expression of these chemokines and the extent of mural inflammation.

Method and Methods

Study design

Studies were performed with the approval of the Leicestershire Research Ethics Committee and written consent was obtained from all participants or next of kin. Full

thickness AAA wall specimens were obtained from the anterior wall at the point of maximal aortic dilation during elective open AAA repair of asymptomatic AAAs. All AAA samples were acquired from infrarenal, atherosclerotic aneurysms [$n = 25$, median age = 73 years (range, 61–84 years), male:female ratio = 24:1 (96%), positive smoking history = 22/25 (88%), median AAA diameter = 64 mm (range, 55–93 mm)]. Patients with inflammatory AAAs or evidence of retroperitoneal fibrosis, symptomatic or ruptured AAAs were excluded.

Control aortic specimens were obtained from: non-aneurysmal abdominal aortic specimens from cadaveric kidney donors provided by the UK Human Tissue Bank (Leicester, UK) [$n = 15$, median age = 54 years (range, 23–74 years), male:female ratio = 11:4 (73%), positive smoking history = 12/15 (80%)], and thoracic aorta, as an example of atherosclerotic aorta, from patients undergoing aorto-coronary bypass graft operations [$n = 10$, median age = 66 years (range, 50–76 years), male:female ratio = 8:2 (80%), positive smoking history = 8/10 (80%)].

Tissue collection

At the point of harvesting the specimens were snap frozen in liquid nitrogen and stored at -80°C until ELISA analysis, or fixed in 10% formalin and embedded in paraffin wax for histological analysis. Where the size of specimen obtained from theatre was limited, the specimen was subjected to ELISA analysis only.

ELISA analysis

IL-8 and MCP-1 expression was measured in 25 AAA specimens compared to 15 abdominal aortic control specimens and 10 thoracic aortic control specimens using enzyme-linked immunosorbent assays (ELISAs). The protein extract of the specimens was prepared by homogenising the specimens according to an established methodology.¹⁸ The total protein concentration of the resulting supernatant was determined using a bicinchoninic acid protein assay method (Pierce, Rockford, USA). All homogenates were standardised to a protein concentration of 1 mg/ml. ELISA kits (BioSource International, Inc., Nivelles, Belgium) were used to quantify IL-8 and MCP-1 concentration within each specimen and performed according to the manufacturer's protocol.

Antibodies

The following antibodies were supplied by (1) R&D Systems, Abingdon, UK: IL-8 (Clone 6217 mouse anti-human IL-8, diluted 1:20), and MCP-1 (Clone 23002 mouse anti-human MCP-1, diluted 1:25); (2) DakoCytomation, Ely, UK: CD3 (T-lymphocytes: polyclonal rabbit anti-human CD3, diluted 1:400), CD20 (B-lymphocytes: Clone L26 mouse anti-human CD20, diluted 1:1000), and CD68 (Macrophages: Clone PG-M1 mouse anti-human CD68, diluted 1:150); (3) Novocastra Laboratories, Newcastle upon Tyne, UK: CD4 (T_H-lymphocytes: Clone 1F6 mouse anti-human CD4, diluted 1:25), and CD8 (T_C-lymphocytes: Clone 1A5 mouse anti-human CD8, diluted 1:200).

Immunohistochemistry

Immunohistochemistry was performed on the AAA specimens ($n = 20$) and control abdominal aorta ($n = 15$). Antigen retrieval was accomplished through heat treatment in 10 mM citrate buffer, pH 6.0 (CD3, CD4, CD8 and CD20), heat treatment in a high pH buffered solution (Target Retrieval Solution, pH 9.0, DakoCytomation) (IL-8), and trypsin digestion in a 0.1% trypsin solution containing 10 mM calcium chloride, pH 7.8 (CD68 and MCP-1). The specimen sections were treated with primary antibody for 1 h at room temperature. Endogenous peroxidase activity was blocked by incubation with a peroxidase blocking solution (DakoCytomation) for 10 min at room temperature. Antibody specific binding was detected using the EnVision Detection System (DakoCytomation) according to the manufacturer's instructions. The resulting complex was detected using a 0.05% solution of 3,3'-diaminobenzidine (DAB) per slide for 10 min at room temperature. Slides were washed in water for 5 min and counterstained in haematoxylin. Serial sections were also stained by haematoxylin and eosin (H&E). Nonrelevant isotype matched monoclonal negative controls and no primary antibody negative controls were also performed.

Microscopic evaluation

The extent of inflammation within a specimen was graded from the H&E stains according to the following scale: 0 = no or only a few inflammatory cells visible, with no or little fibrosis and no loss of smooth muscle cells (SMCs); 1 = mild inflammation with diffused inflammatory cells, with mild fibrosis of the media and mild loss of SMCs; 2 = moderate inflammation with the appearance of dense follicles (1–3 follicles), with medial degeneration and notable absence of SMCs; 3 = extensive inflammation with ≥ 4 follicles or such extensive infiltration that the follicles have merged, severe fibrosis and severe loss of SMCs.¹⁹ Lymphocytes ((Cluster of Differentiation) CD3, CD4, CD8 and CD20) were graded based on the percentage of positively stained cells within the infiltrate.⁴ Grading was scored as follows: 0 = 0%, 1 = 2–10%, 2 = 10–25%, 3 = 25–50%, 4 = 50–75%, 5 = 75–100%. CD68, IL-8 and MCP-1 stainings were scored according to the extent of staining present under a high-powered field of view. Scores ranged from 0 to 3, with 0 representing no staining through to 3 representing extensive staining.⁴ Specimens between grades were given as the mean of the two grades. The results were independently assessed by two observers, including a pathologist.

Statistical analysis

Data are presented as medians with interquartile ranges. Statistical analysis of continuous data was performed using a Kruskal–Wallis test, followed by a series of Mann–Whitney U tests. Mann–Whitney tests were corrected for multiple comparisons by the Bonferroni method using an adjusted P value of $< .017$ to determine statistical significance for the Mann–Whitney tests. Categorical associations were determined using a Chi-square test and correlations

were assessed using Spearman's rank correlation. P value of $< .05$ was used to determine a significant difference.

Results

IL-8 and MCP-1 concentration in AAA

Fig. 1A shows the concentration of IL-8 expressed within the wall of the AAA, and non-aneurysmal abdominal and thoracic aorta, as measured by ELISAs. IL-8 expression was significantly increased within the AAA (438.6 pg/ml [interquartile range, 174.6 pg/ml–1063.5 pg/ml]) compared with non-aneurysmal abdominal aorta (< 15.6 pg/ml [interquartile range, < 15.6 pg/ml–74.4 pg/ml]) ($P < .001$) and non-aneurysmal thoracic aorta (< 15.6 pg/ml [interquartile range, < 15.6 pg/ml– < 15.6 pg/ml]) ($P < .001$). There was no statistical difference in the concentration of IL-8

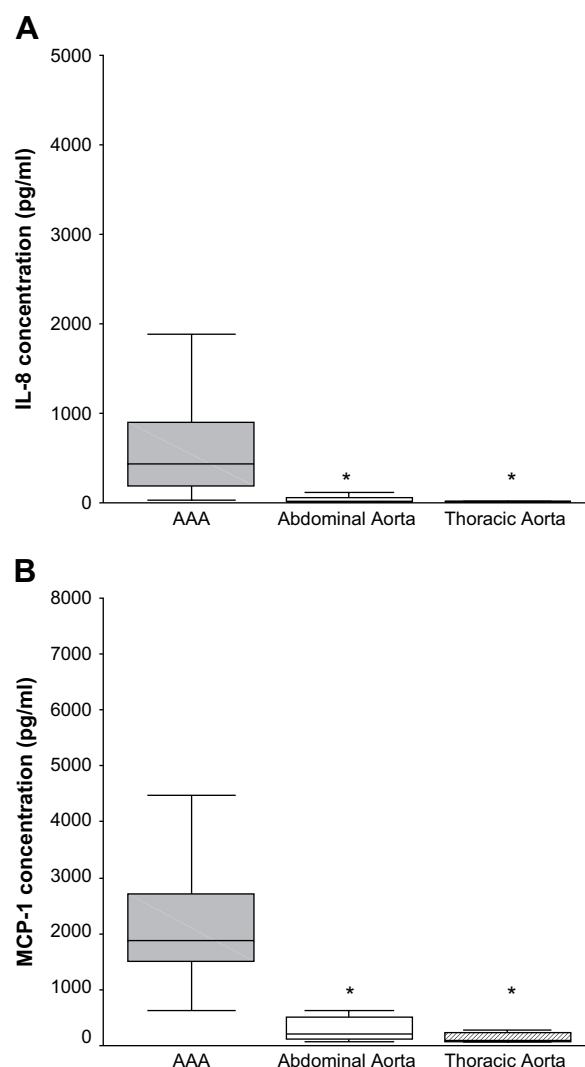


Figure 1 IL-8 (A) and MCP-1 (B) expression within AAA ($n = 25$), non-aneurysmal abdominal aorta ($n = 15$), and non-aneurysmal thoracic aorta ($n = 10$). An asterisk (*) above a box represents a significant difference between that non-aneurysmal group and the AAA group ($P < .001$).

between the non-aneurysmal abdominal and thoracic aorta ($P = .061$). Whilst there was a difference in age between the AAA specimens and the non-aneurysmal abdominal and thoracic aorta, there was no correlation between IL-8 or MCP-1 expression and age for the AAA, abdominal and thoracic aorta groups (Spearman's rank correlation: IL-8: AAA $P = .212$, abdominal aorta $P = .413$ and thoracic aorta $P = .241$).

Fig. 1B shows the concentration of MCP-1 expressed within the AAA wall, and non-aneurysmal abdominal and thoracic aorta. MCP-1 expression was also significantly increased within the AAA (1869.1 pg/ml [interquartile range, 1496.6 pg/ml–2713.4 pg/ml]) compared with non-aneurysmal abdominal aorta (198.8 pg/ml [interquartile range, 99.6 pg/ml–621.5 pg/ml]) ($P < .001$) and non-aneurysmal thoracic aorta (96.9 pg/ml [interquartile range, 61.5 pg/ml–238.6 pg/ml]) ($P < .001$). Based on the

adjusted P value used to determine significance, there was no statistical difference in the concentration of MCP-1 between the non-aneurysmal abdominal and thoracic aorta ($P = .047$). There was no correlation between MCP-1 expression and age for the AAA, abdominal and thoracic aorta groups (Spearman's rank correlation: AAA $P = .132$, abdominal aorta $P = .253$ and thoracic aorta $P = .282$).

Characterisation of the local inflammatory infiltrate

The predominant cell type within the infiltrate of the AAA was the CD20⁺ B-lymphocyte (Fig. 2A), which were usually present in dense follicles within the adventitia. Occasionally, in cases of severe inflammation, B-lymphocytes were found in the media. There were very few B-lymphocytes observed within the non-aneurysmal abdominal aortic wall.

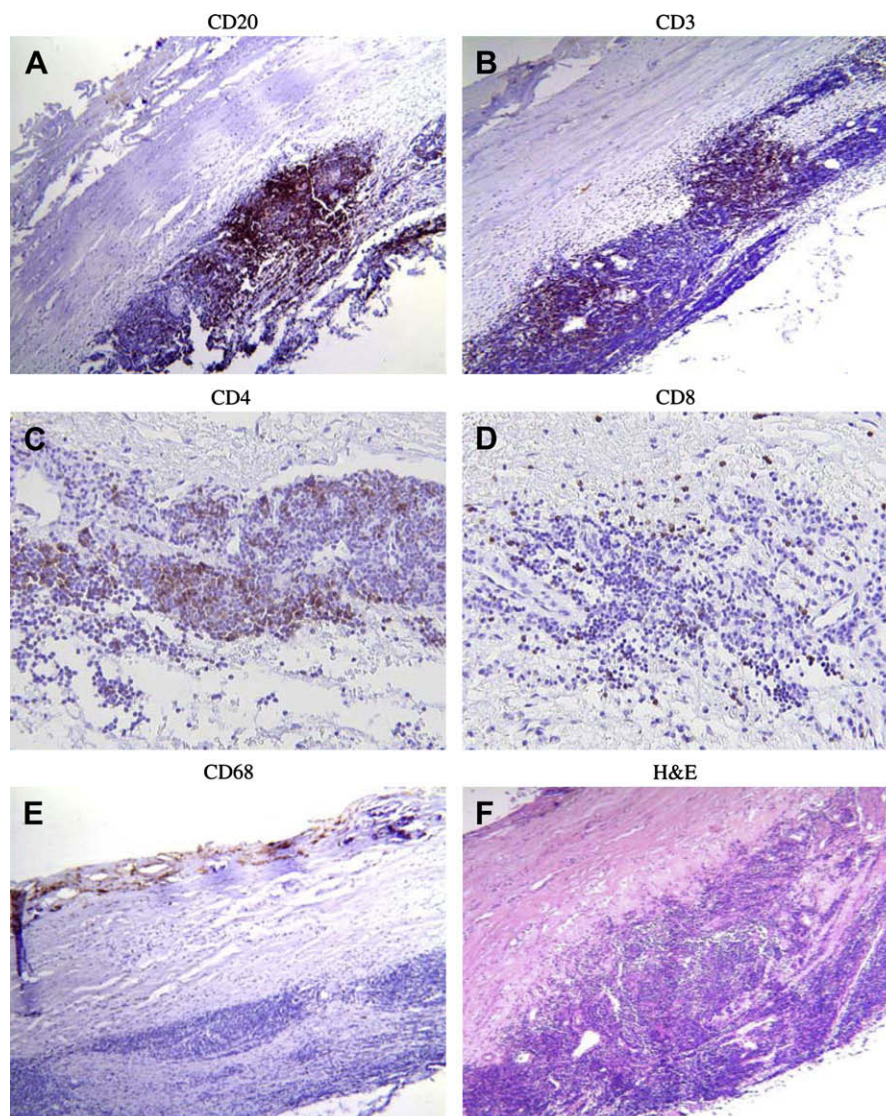


Figure 2 Immunohistochemistry analysis of the inflammatory infiltrate within the AAA (A–F). Sections were stained for CD20⁺ B-lymphocytes (A), CD3⁺ T-lymphocytes (B), CD4⁺T_H-lymphocytes (C), CD8⁺T_C-lymphocytes (D), CD68⁺ macrophages (E), and stained with haematoxylin & eosin (H&E) (F). The cross-sections are orientated with intima at the top and adventitia at the bottom of the image (magnification: $\times 40$). C and D depict the adventitial layer only (magnification: $\times 250$).

CD3⁺ T-lymphocytes were prevalent within the infiltrate, although to a lesser extent than B-lymphocytes. The T-lymphocytes were found in follicles, or dispersed throughout the adventitial layer in milder inflammatory conditions (Fig. 2B). The T-lymphocytes were also dispersed through the media in cases of severe inflammation. Lymphocytes were only occasionally present within the non-aneurysmal abdominal aortic wall, and were usually of the CD3⁺ phenotype, found in the intimal and occasionally medial layers. The T-lymphocyte population within both the AAA and the non-aneurysmal abdominal aorta consisted predominantly of CD4⁺ T_H-lymphocytes with the occasional scattered appearance of CD8⁺ T_C-lymphocytes (Fig. 2C and D).

CD68⁺ macrophages were predominantly found within the intima of the AAA (Fig. 2E). Macrophages were rarely seen in the media, however, solitary macrophages were occasionally present within the adventitia. Macrophages

were the principal inflammatory cell type in the non-aneurysmal abdominal aorta. Low levels of these macrophages were occasionally found within the intima. However, macrophages were rarely found within the adventitia of the non-aneurysmal abdominal aorta.

Compared to non-aneurysmal abdominal aorta, the AAA wall structurally showed medial degradation with a loss of SMCs, and a thickening of the adventitia. The most notable feature in Fig. 2F is the intense infiltration of the adventitia by inflammatory cells.

Localised expression of IL-8 and MCP-1 within the AAA wall

Immunohistochemical staining revealed that IL-8 was associated with the inflammatory infiltrate and was predominantly expressed within the adventitial layer (Fig. 3A and C).

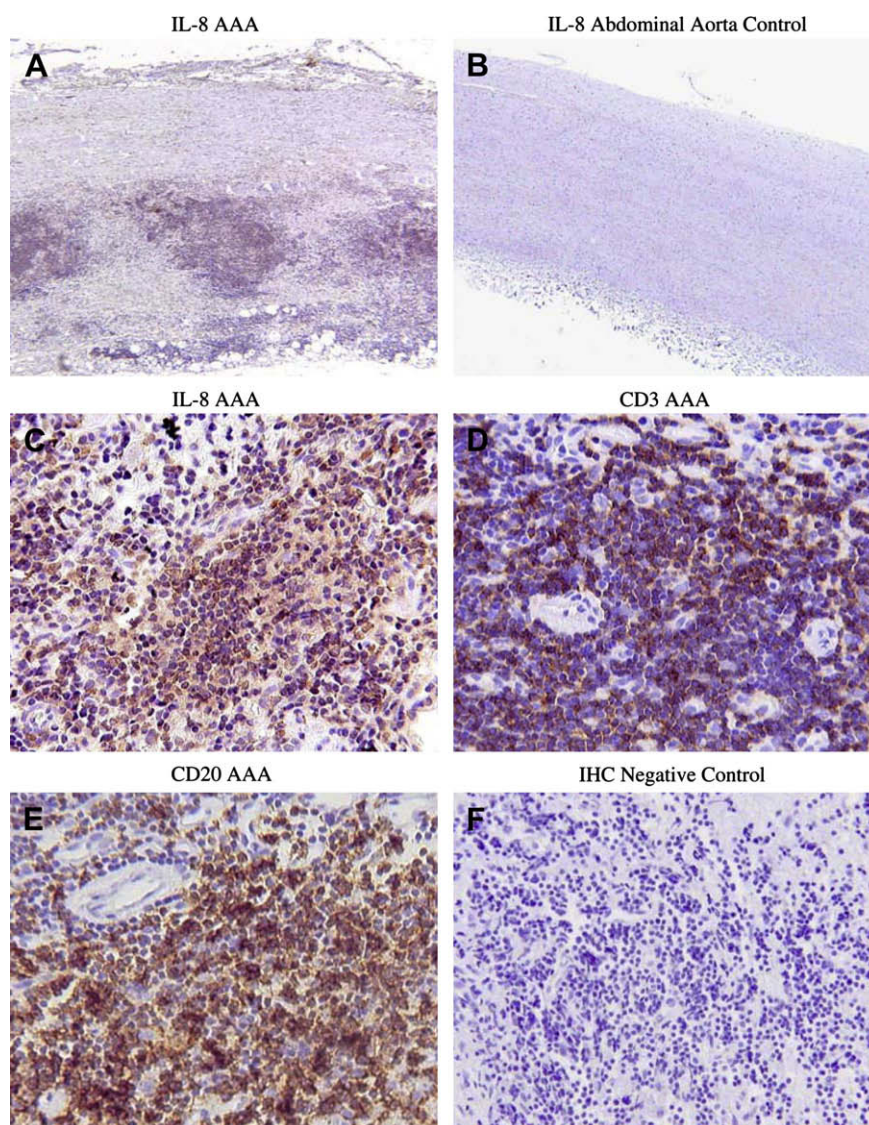


Figure 3 IL-8 expression within the AAA (A) and the non-aneurysmal abdominal aorta (B). The localisation of IL-8 to the infiltrate within the adventitia (C) and the presence of CD3⁺ T-lymphocytes (D) and CD20⁺ B-lymphocytes (E) within the infiltrate. The immunohistochemistry (IHC) negative control (F). The cross-sections are orientated with intima at the top and adventitia at the bottom of the image (magnification: $\times 40$). C–F depict the adventitial layer only (magnification: $\times 250$).

The non-aneurysmal abdominal aorta was negative for IL-8 expression (Fig. 3B). IL-8 expression was localised to the follicles within the adventitia, which were predominantly formed from B- and T-lymphocytes (Fig. 3C–E).

MCP-1 expression was predominantly found within the intima of the AAA specimens (Fig. 4A). Macrophages were also predominantly found within the intima, with a similar staining pattern as for MCP-1 (Fig. 4B). Solitary MCP-1 positive macrophages were occasionally observed within the media and adventitia of the AAA (Fig. 4C and D). Fig. 4E and F represents typical staining for MCP-1 and CD68⁺

macrophages, respectively, within the non-aneurysmal abdominal aorta. Occasionally MCP-1 staining was observed in the intima and was associated with macrophages within the non-aneurysmal abdominal aorta.

The association of AAA inflammation with the expression of IL-8 and MCP-1

AAA specimens were grouped into three categories based on the severity of the IL-8 and MCP-1 staining. The medical history of the AAA patients in each group is shown in

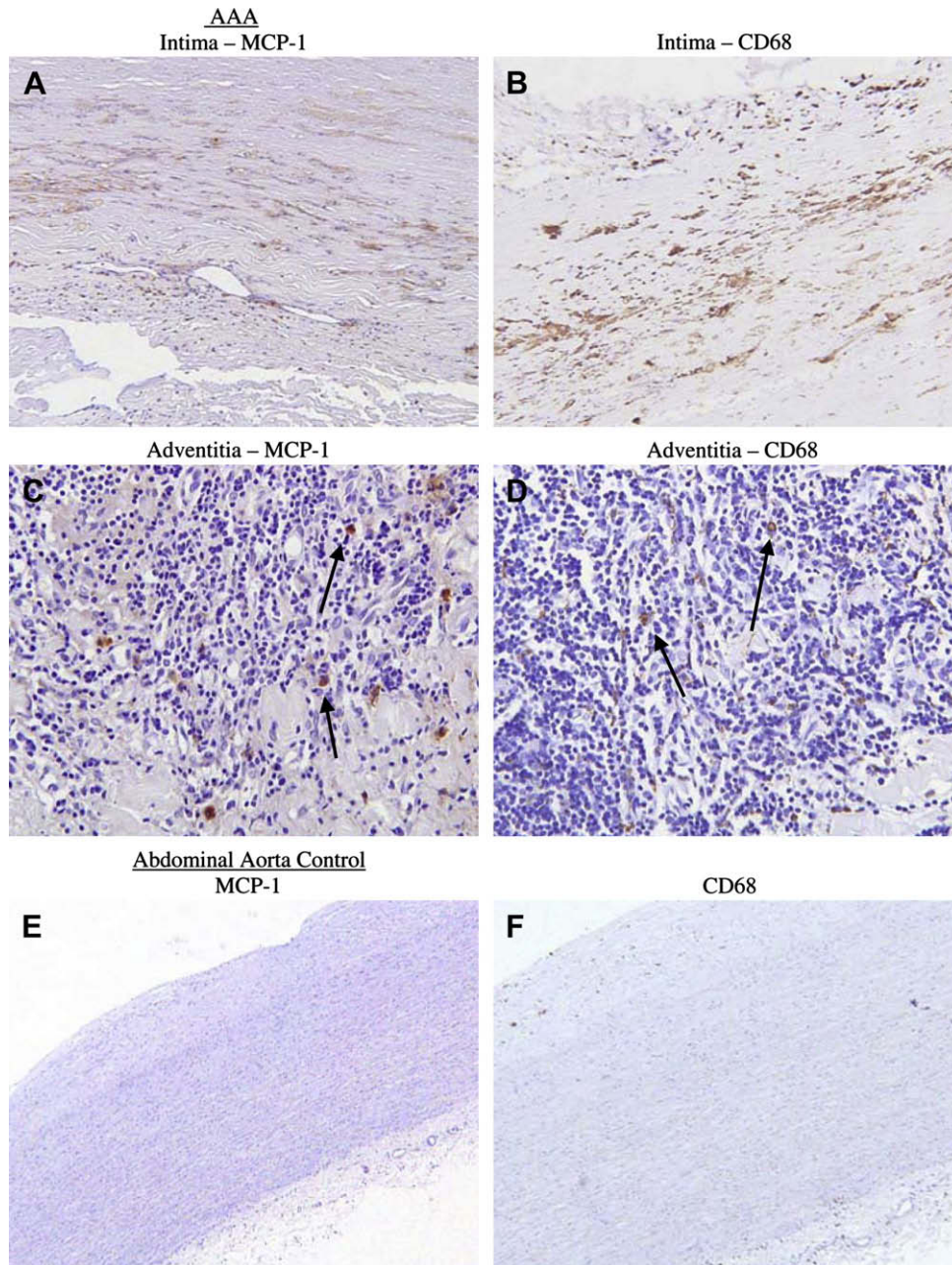


Figure 4 Localisation of MCP-1 expression and CD68⁺ macrophages within the AAA to the intima (A and B, respectively) and adventitia (C and D, respectively). Staining of the non-aneurysmal abdominal aorta for MCP-1 and CD68⁺ macrophages (E and F, respectively). The cross-section is orientated with the intima at the top and adventitia at the bottom of the image (magnification: A and B $\times 100$; C and D $\times 250$; E and F $\times 40$).

Table 1 Medical history of the AAA population grouped according to IL-8 expression

Medical history	Grade 1 – mild	Grade 2 – moderate	Grade 3 – severe
	IL-8 (<i>n</i> = 10)	IL-8 (<i>n</i> = 6)	IL-8 (<i>n</i> = 4)
Median age (IQR)/years	74 (67–78)	70 (66–74)	76 (68–81)
Male sex, <i>n</i> (%)	9 (90)	6 (100)	4 (100)
Smoking history, <i>n</i> (%)	9 (90)	6 (100)	2 (50)
Median AAA diameter (IQR)/mm	67 (56–73)	61 (57–75)	79 (68–92)
Aspirin, <i>n</i> (%)	6 (60)	3 (50)	3 (75)
Beta-blockers, <i>n</i> (%)	4 (40)	1 (17)	1 (25)
ACE inhibitors, <i>n</i> (%)	4 (40)	2 (33)	1 (25)
Warfarin, <i>n</i> (%)	1 (10)	0 (0)	0 (0)
Statins, <i>n</i> (%)	3 (30)	2 (33)	0 (0)

Tables 1 and 2. The median H&E inflammatory score of each IL-8 group was determined and specimens with an extensive expression of IL-8 were associated with a severe inflammation of the AAA wall (Chi-square value = 9.862, 4 d.f., $P = .043$) (Table 3). Overall, the level of mural inflammation increased with increasing IL-8 expression (median H&E score for mild IL-8 group: 1.5 [interquartile range, 1–2] versus median H&E score for severe IL-8 group: 3 [interquartile range, 3–3]). Table 3 also shows the results of grouping the AAA specimens based on the level of MCP-1 expression and the median inflammation within each category. No association was found between MCP-1 expression and the level of inflammation (Chi-square value = 3.960, 4 d.f., $P = .411$).

A comparison of the size of AAAs for each IL-8 group demonstrated that AAAs were significantly larger in specimens with a severe level of IL-8 expression compared to a mild or moderate level of expression (median AAA size for mild IL-8 group: 67 mm [interquartile range, 56–73 mm] versus median AAA size for severe IL-8 group: 79 mm [interquartile range, 68–92 mm] (Kruskal–Wallis, $P < .001$) (Fig. 5A and Table 3). There was no significant difference in the size of AAAs between the three categories based on MCP-1 expression (Kruskal–Wallis, $P = .982$) (Fig. 5B and Table 3).

The association between IL-8 and the inflammatory infiltrate composition

The inflammatory infiltrate composition of the three AAA groups, categorised by the severity of IL-8 expression,

revealed that increasing IL-8 expression was associated with a significant increase in CD3⁺ T-lymphocytes (Kruskal–Wallis, $P = .013$) of the CD4⁺ phenotype (Kruskal–Wallis, $P = .012$) (Table 4). Mann–Whitney *U* analysis of paired IL-8 groups revealed that there was a significant difference between the mild and severe IL-8 groups for CD3 (mild median CD3 score: 1.5 [interquartile range, 0.5–2] versus severe median CD3 score: 4 [interquartile range, 3.5–4.5], $P = .005$), and CD4 (mild median CD4 score: 0.75 [interquartile range, 0–1] versus severe median CD4 score: 3.5 [interquartile range, 2.5–4.5], $P = .005$). The number of CD8⁺T_C-lymphocytes within the infiltrate did not alter with IL-8 expression ($P = .447$), neither did CD20⁺ B-lymphocytes ($P = .263$) nor CD68⁺ macrophages ($P = .085$) (Table 4).

Discussion

Chemokine expression has been overlooked within the AAA with few studies focusing on the potential role of chemokines within AAA pathogenesis. The potential involvement of these proteins in AAA pathogenesis has been recently highlighted by our study on 42 cytokines which showed the prominent increased expression of several members of the chemokine family within the AAA.¹² The finding that IL-8 and MCP-1 are highly elevated within the AAA corroborates our previous findings and is supported by several other studies. Previously, both IL-8 and MCP-1 have been shown to be increased in the AAA at the protein level compared to occlusive and normal aorta,¹¹ and an 11-fold increase in

Table 2 Medical history of the AAA population grouped according to MCP-1 expression

Medical history	Grade 1 – mild	Grade 2 – moderate	Grade 3 – severe
	MCP-1 (<i>n</i> = 10)	MCP-1 (<i>n</i> = 8)	MCP-1 (<i>n</i> = 2)
Median age (IQR)/years	73 (66–79)	73 (67–77)	73 (71–74)
Male sex, <i>n</i> (%)	9 (90)	8 (100)	2 (100)
Smoking history, <i>n</i> (%)	8 (80)	7 (88)	2 (100)
Median AAA diameter (IQR)/mm	68 (61–74)	67 (56–88)	65 (60–70)
Aspirin, <i>n</i> (%)	6 (60)	5 (63)	1 (50)
Beta-blockers, <i>n</i> (%)	2 (20)	4 (50)	0 (0)
ACE inhibitors, <i>n</i> (%)	3 (30)	4 (50)	0 (0)
Warfarin, <i>n</i> (%)	1 (10)	0 (0)	0 (0)
Statins, <i>n</i> (%)	2 (20)	3 (38)	0 (0)

Table 3 Comparison between the level of inflammation within the AAA wall, AAA size and increasing levels of IL-8/MCP-1 expression

Parameter	Median Score (IQR) for AAAs grouped based on IL-8/MCP-1 staining			P value
	Grade 1 – mild	Grade 2 – moderate	Grade 3 – severe	
	IL-8 (n = 10)	IL-8 (n = 6)	IL-8 (n = 4)	
H&E	1.5 (1–2)	1 (1–2)	3 (3–3)	.043 ^a
AAA diameter	67 (56–73)	61 (57–75)	79 (68–92)	<.001 ^b
Parameter	Median Score (IQR) for AAAs grouped based on IL-8/MCP-1 staining			P value
	Grade 1 – mild	Grade 2 – moderate	Grade 3 – severe	
	MCP-1 (n = 10)	MCP-1 (n = 8)	MCP-1 (n = 2)	
H&E	2.5 (1–3)	1.5 (1–2.5)	1 (1–1)	.411 ^a
AAA diameter	68 (61–74)	67 (56–88)	65 (60–70)	.982 ^b

^a Chi-square.^b Kruskal–Wallis test.

IL-8 mRNA expression in AAAs compared to normal aorta has been described.²⁰ The IL-8 receptor, CXCR2, has also been shown to be increased within human AAAs,²¹ suggesting that a pathway involving IL-8 signalling may be involved in AAA pathogenesis.

This study found that IL-8 was localised to the lymphocyte population within the adventitia of the AAA, whereas

the majority of the non-aneurysmal abdominal aortas were negative for IL-8. This further supports the involvement of IL-8 in aneurysm pathogenesis. The only other study on IL-8 expression within the aneurysm wall, however, reported that macrophages were positive for IL-8 and to a lesser extent endothelial cells.¹¹ It is possible that positive macrophage expression of IL-8 within the infiltrate was masked by the overwhelming positive staining of the lymphocytes, but IL-8 staining was rarely found within the intima, which was the main source of macrophages within the AAAs studied.

The MCP-1 positive cells were identified as being predominantly macrophages, as previously reported.¹¹ Within the AAA the MCP-1 positive staining localised to the intima and adventitia, mirroring the staining pattern of CD68⁺ macrophages. The expression of MCP-1 within the adventitia of the AAA suggests that MCP-1 may also be involved in the pathogenesis of the AAA. A potential role for MCP-1 in AAA formation is supported by evidence from an angiotensin II induced AAA model (in Apo E^{-/-} deficient mice) where those mice with the additional knockout of CCR2, the receptor for MCP-1, suppressed the formation of AAAs compared to those with CCR2.²²

The exact role IL-8 and MCP-1 may contribute to AAA pathogenesis is unknown. It has been demonstrated that IL-8 is chemotactic for human aortic endothelial cells.¹⁶ IL-8 also can induce cellular expression of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)²³ and may have a role in leukocyte chemotaxis. MCP-1 has been shown to be up-regulated within the AAA wall of an elastase-induced mouse model prior to macrophage infiltration of the aortic wall.²⁴ Furthermore, MCP-1 can also up-regulate the expression of CD11b and CD11c integrins on monocytes, enabling the monocytes to interact with endothelial cells during inflammation.^{25,26} Therefore increased IL-8 and MCP-1 within the AAA may have a role in the development and or maintenance of the inflammatory infiltrate.

This study found that the level of mural inflammation increased with increasing IL-8 expression and that a high level of IL-8 expression was associated with an increase in AAA size. No such relationship was determined for MCP-1. However, as few AAA specimens had an immunohistological grading of severe it may be that it wasn't possible to detect

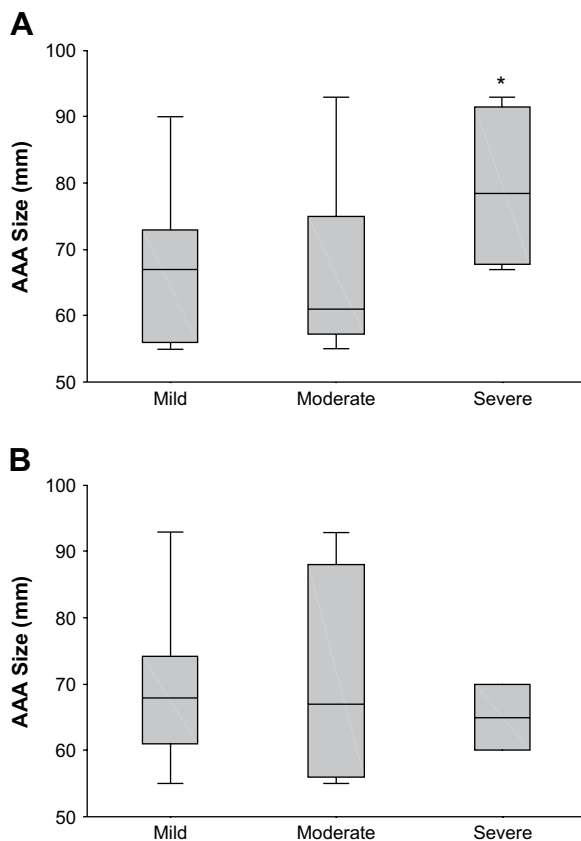


Figure 5 Size of AAAs within the IL-8 (A) and MCP-1 (B) groups. AAAs were grouped according to IL-8/MCP-1 expression as mild, moderate and severe (based on IHC staining). The asterisk (*) represents a significant difference between the severe IL-8 group and the mild and moderate IL-8 groups ($P < .001$).

Table 4 Comparison of adventitial infiltrate composition at different levels of IL-8 expression

CD staining	CD Median Score (IQR) for each IL-8 Grade			Kruskal–Wallis test	Mann–Whitney <i>U</i> test
	IL-8 Grade 1 Mild ^a	IL-8 Grade 2 Moderate ^b	IL-8 Grade 3 Severe ^c	<i>P</i> value	<i>P</i> value Grade 1 vs 3
CD3	1.5 (0.5–2)	2 (1–3)	4 (3.5–4.5)	.013	.005
CD4	0.75 (0–1)	1 (0.5–2.5)	3.5 (2.5–4.5)	.012	.005
CD8	0.5 (0–1)	0.5 (0–1)	1 (0.75–1.5)	.447	n/a
CD20	3 (2.5–4)	4 (3.5–4)	2.75 (2.25–3.5)	.263	n/a
CD68	0.25 (0–1)	0 (0–1)	1.5 (1–2.5)	.085	n/a

^a *n* = 10.^b *n* = 6.^c *n* = 4.

an association. IL-8 and MCP-1 frequently interact synergistically²⁷ and therefore the presence of MCP-1 may augment the chemotactic response to IL-8. Whilst the lymphocyte population stained positive for IL-8, it wasn't possible to determine whether these were B- or T-lymphocytes. However, examination of the association between IL-8 and the composition of the infiltrate revealed a significant increase in T-lymphocytes, including T_H-lymphocytes, with increasing IL-8 expression. This suggests that IL-8 may have a chemotactic role in T_H-lymphocyte recruitment within the aneurysm wall which could promote further inflammation and cytokine release within the aneurysm wall.

A limitation of this study was that the AAA specimens were obtained at surgery and therefore were established aneurysms. This limits the results to an interpretation of the state of the developed aneurysm wall which is undergoing expansion and at risk of rupture, but cannot be used to determine the earlier stages of aneurysm formation. The relationship between severe IL-8 expression and large AAA size supports the involvement of this chemokine in late stage AAA development. This study is relatively small, and whilst the results show a potential involvement of IL-8 in the development of the infiltrate the small study size means it is possible that the statistical tests used were of low power. Future work on receptor expression is required to further elucidate possible mechanisms for the action of IL-8 within the AAA wall.

In summary, these findings support the involvement of IL-8 and MCP-1 in the pathogenesis of AAAs and, whilst the role of MCP-1 is unclear, the results suggest that IL-8 may have a role in the recruitment of T_H-lymphocytes into the AAA wall. Further investigations are required to fully elucidate the chemotactic role of IL-8 and MCP-1 within the AAA wall.

Conflict of Interests

The authors have no conflicts of interest to disclose.

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